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Title Page

Evaluation of 89 compounds for identification of substrates for cynomolgus monkey cytochrome P450 2C76, a new bupropion/nifedipine oxidase

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Running Title Page

Running title: Novel CYP2C76 substrates

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Abbreviations: BA, bioavailability ; CLint, intrinsic clearance ; LC, liquid chromatography; MS/MS, tandem mass spectrometry ; P450, cytochrome P450 (EC 1.14.14.1).

Abstract

Cynomolgus monkeys are widely used in preclinical studies during drug development because of their evolutionary closeness to humans, including cytochrome P450 (P450 or CYP). Most cynomolgus monkey P450s are almost identical (≥90%) to human P450s; however, CYP2C76 has low sequence identity (approximately 80%) to any human CYP2Cs. Although CYP2C76 has no ortholog in humans and is partly responsible for species difference in drug metabolism between cynomolgus monkeys and humans, a broad evaluation of potential substrates for CYP2C76 has not yet been conducted. In this study, a screening of 89 marketed compounds, including human CYP2C and non-CYP2C substrates or inhibitors, was conducted to find potential CYP2C76 substrates. Among the compounds screened, 19 chemicals were identified as substrates for CYP2C76, including substrates for human CYP1A2 (7-ethoxyresorufin), CYP2B6 (bupropion), CYP2D6 (dextromethorphan), and CYP3A4/5 (dextromethorphan and nifedipine), and inhibitors for CYP2B6 (sertraline, clopidogrel, and ticlopidine), CYP2C8 (quercetin), CYP2C19 (ticlopidine and nootkatone), and CYP3A4/5 (troleandomycin). CYP2C76 metabolized a wide variety of the compounds with diverse structures. Among them, bupropion and nifedipine showed high selectivity to CYP2C76. As for nifedipine, CYP2C76 formed methylhydroxylated nifedipine, which was not produced by monkey CYP2C9, CYP2C19, or CYP3A4, as identified by mass spectrometry and estimated by a molecular docking simulation. This unique oxidative metabolite formation of nifedipine could be one of the selective marker reactions of CYP2C76 among the major CYP2C and CYP3A tested. These results suggest that monkey CYP2C76 contributes to bupropion hydroxylation and formation of different nifedipine oxidative metabolite(s) due to its relatively large substrate cavity.

Introduction

Due to their evolutionary closeness and physiological resemblance to humans, cynomolgus monkeys have been widely used in various biomedical research studies including neuroscience and reproduction physiology. Age-related alterations of physiological parameters in monkeys are in agreement with clinical observations in humans, suggesting that monkeys might be a suitable animal model for prediction of age-related changes in pharmacokinetics in humans (Koyanagi et al., 2014). Although monkeys are frequently used in preclinical studies for drug development, the differences between monkeys and humans in pharmacokinetic profile are occasionally seen.

The cytochrome P450 (P450 or CYP) superfamily consists of a large number of drug-metabolizing enzyme genes. P450s play a critical role in metabolism of drugs, steroids, fatty acids, and environmental pollutants. In humans, the CYP1-3 families are important for drug metabolism, whereas the CYP4 family is mainly involved in metabolism of endogenous substrates and, to a lesser extent, drugs. To date, more than 20 P450 isoforms have been identified in cynomolgus monkeys (Uno et al., 2011a). It should be noted that P450s of primate species such as cynomolgus monkeys are evolutionarily much closer to those of humans than those of dogs, rats, or mice, which are commonly used in drug metabolism studies and preclinical toxicity tests.

Most cynomolgus monkey P450 cDNAs have high sequence identities (≥ 90%) to human P450 cDNAs; however, CYP2C76 cDNA has only 75-78% sequence identity to human CYP2C cDNAs (Uno et al., 2006). In addition, CYP2C76 has no ortholog in humans (Uno et al., 2006; Uno et al., 2010), raising the possibility that CYP2C76 is involved in species differences in drug metabolism between cynomolgus monkeys and humans. CYP2C76 protein is expressed in liver, and catalyzes tolbutamide 4-hydroxylation and testosterone

2α-/16α-hydroxylation, but not paclitaxel 6α-hydroxylation or *S*-mephenytoin 4'-hydroxylation, showing substrate specificity differences to those of other cynomolgus monkey and human CYP2Cs (Uno et al., 2006). CYP2C76 also metabolizes non-CYP2C substrates in humans such as 7-ethoxyresorufin and bufuralol (Uno et al., 2011b). Pitavastatin is metabolized largely by monkey CYP2C19 and CYP2C76, but CYP2C76 catalyzes the same reaction catalyzed by CYP3A in humans and is also involved in the formation of metabolites not found in humans (Uno et al., 2007). These facts suggest that CYP2C76 is responsible for the differences of drug metabolism between monkeys and humans. However, a broad evaluation of potential substrates for CYP2C76 has not yet been conducted to understand the substrate specificity of this isoform.

In this study, 89 marketed compounds, including human CYP2C and non-CYP2C substrates or inhibitors (Rendic, 2002), also found in Food and Drug Administration Drug-Drug Interaction Draft Guidance, 2006, (http://www.fda.gov/cder/guidance/6695dft.htm) and European Medicines Agency (EMA) guidelines (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf), were screened as potential CYP2C76 substrates. From the compounds screened, 19 chemicals were identified as substrates for CYP2C76, and these structures had wide diversity, from relatively small molecule (e.g. nootkatone) to larger molecule (e.g. troleandomycin) compounds. Among the newly identified substrates, CYP2C76 metabolized nifedipine, forming a metabolite unique to cynomolgus monkeys, and thus this could be a selective marker reaction of CYP2C76 among a variety of monkey CYP2C and CYP3A mediated reactions. We report herein that CYP2C76 has relatively wide substrate specificity and ability to form unique metabolites, which could result in species differences of drug oxidation between monkeys and humans.

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Materials and Methods

Materials

Nifedipine and bupropion were purchased from Wako (Osaka, Japan). Pitavastatin lactone and dehydronifedipine were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The other drugs in Table 1 were obtained from one of the following sources: Wako (Osaka, Japan), Sigma-Aldrich (St. Louis, MO), Cosmo Bio (Tokyo, Japan), or Nacalai Tesque (Kyoto, Japan). Cynomolgus monkey P450 recombinant enzymes, CYP2C76, CYP2C9, CYP2C19, CYP2B6, and CYP3A4, were expressed in *Escherichia coli* and subsequently purified as described previously (Iwata et al., 1998; Daigo et al., 2002). Other reagents used in this study were of the highest quality commercially available.

Oxidations of nifedipine, bupropion, and other possible monkey P450 substrates

The substrate was 1 or 20 μM dissolved in final concentrations of 0.01-0.02% DMSO or 1% methanol. Incubation mixtures contained substrate, 10-25 pmol/mL recombinant cynomolgus monkey P450, 0.25 mM β-$NADP^+$, 2.5 mM glucose 6-phosphate, 0.025 U glucose-6-phosphate dehydrogenase, and 30 mM magnesium chloride in a final volume of 100 μL of 50 mM potassium phosphate buffer, pH 7.4. After incubation at 37°C for 0-30 min, reactions were terminated by addition of 200 μL of ice-cold methanol containing 5 nM terbinafine or 300 nM mefenamic acid as internal standards. Samples were then centrifuged at 21,600×g for 10 min, and the supernatants were analyzed by LC-MS/MS. All incubations were performed in duplicate.

LC-MS analytical methods

The LC system consisted of a pump and a Nanospace SI-2 autosampler (Shiseido, Tokyo,

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Japan) using an analytical C_{18} reversed-phase column (CAPCELL CORE C_{18}, 2.1×50 mm, 2.7 μm; Shiseido) with a TSQ QUANTUM ULTRA triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The injection volume was 2-10 μL. LC conditions were as follows: solution A contained 0.1% formic acid (v/v) in water and solution B contained 0.1% formic acid (v/v) in methanol. The following gradient program was used at a flow rate of 0.40 mL/min: 0–0.1 min, linear gradient from 98% A to 0% A (v/v); 0.1–1.6 min, hold at 0% A; 1.6–1.7 min, linear gradient from 0% A to 98% A (v/v); 1.7–4.2 min, hold at 98% A. After electrospray ionization, compounds were analyzed by selected reaction monitoring mode. The peak area ratio of the analyte to the internal standard was determined for each injection and used to measure substrate depletion. In the chromatographic analysis of nifedipine and bupropion metabolites, CAPCELL PAC C18 ACR column (2.0×150 mm, 5 μm; Shiseido) and the following gradient program were used at a flow rate of 0.40 mL/min: 0–3 min, hold at 98% A; 3–50 min, linear gradient from 98% A to 0% A (v/v); 50–55 min, hold at 0% A; 55–55.1 min, linear gradient from 0% A to 98% A (v/v); 55.1–60 min, hold at 98% A. Single MS scan was used to monitor the metabolites.

Data analysis

Residual ratios at 30 min after incubation of each substrate were calculated and converted to substrate disappearance ratios by the following equation:

Substrate disappearance ratio (%) = 100 - Residual ratio (%)

In vitro intrinsic clearance (CL_{int}) was determined by the following equation, where *k* is the slope of the linear regression from the log concentration vs. incubation time (for 0, 15, and 30 min) relationships.

CL_{int} (mL/min/nmol P450) = *k* (/min) × (mL incubation) / (nmol P450)

Docking simulation

Cynomolgus monkey CYP2C76 primary sequence was aligned with a crystal structure of human CYP2C9 (Protein Data Bank code 1R90) (Wester et al., 2004) using MOE software (ver. 2013.10, Computing Group, Montreal, Canada) for modeling of the three-dimensional structure. Prior to docking simulation, the energy of the P450 structures was minimized using the CHARM22 force field. Docking simulation was carried out for nifedipine binding to P450 enzymes using the MMFF94x force field distributed in the ASE Dock software. Solutions were generated for each docking experiment and ranked according to the total interaction energy (*U* value).

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Results

CYP2C76 metabolism of 89 compounds

A total of 89 compounds (Table 1) were evaluated for their potential to undergo metabolism by CYP2C76 and other monkey P450s (CYP2C9, CYP2C19, and CYP3A4). Pitavastatin lactone served as positive control, due to the important role of CYP2C76 for monkey-specific metabolism of pitavastatin lactone (Uno et al., 2007). From the results of substrate depletion assay, 19 compounds showed relatively rapid metabolism with CYP2C76 (substrate disappearance ratios > 20%) (Fig. 1A). Most of these compounds were also metabolized by monkey CYP2C9 and/or CYP2C19; however, nifedipine and bupropion exhibited higher selectivity to CYP2C76 (Fig. 1B). Because nifedipine (Guengerich et al., 1986) and bupropion (Hesse et al., 2000) are known as substrates for CYP3A4 and CYP2B6 in humans, respectively, metabolic activity toward the corresponding monkey P450s were also evaluated. CL_{int} values are shown in Table 2. In monkeys, nifedipine was also metabolized by CYP3A4, and the CL_{int} value was higher than that of CYP2C76. For bupropion, which was also metabolized by monkey CYP2B6, the CL_{int} value was lower than that of CYP2C76. Compared to pitavastatin lactone, the CL_{int} values of nifedipine and bupropion metabolized by CYP2C76 were 10-fold higher and similar, respectively.

Metabolite profiling of nifedipine and bupropion

Because nifedipine and bupropion showed relatively higher selectivity to CYP2C76, their metabolites were further investigated by LC-MS. Figure 2 shows the extracted ion chromatograms of the metabolites observed for nifedipine and bupropion. For nifedipine, $[M-H]^-$ ion of a CYP2C76-specific metabolite was detected at *m/z* 361 (Fig. 2A). In addition, $[M+H]^+$ ion of another nifedipine metabolite at *m/z* 345 was observed with monkey

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CYP3A4, and this metabolite was also detected with CYP2C76 (Fig. 2A). For bupropion, $[M+H]^+$ ion at *m/z* 256 was formed by both CYP2C76 and monkey CYP2B6 (Fig. 2B). An $[M-H]^-$ ion of new nifedipine metabolite at *m/z* 361 and $[M+H]^+$ ion of bupropion metabolite at *m/z* 256 were 16 Da greater than the parent compounds, suggesting that these were oxidative metabolites of the parent compounds. An $[M+H]^+$ ion of known nifedipine metabolite at *m/z* 345 was 2 Da lower than the parent compound, suggesting that this metabolite was formed by dehydrogenation of the 1,4-dehydropyridine ring of nifedipine. To obtain structural information of a new nifedipine metabolite, mass spectrum analysis was also conducted by LC-MS in product ion scan mode. Nifedipine and its oxidative metabolite generated the same fragment ion of *m/z* 122, indicating inclusion of a nitrobenzene unit in their structures (Fig. 3A). The product ions of *m/z* 222 and *m/z* 238 observed in nifedipine and its oxidative metabolite under the present conditions, respectively, indicated that the other unit of nifedipine (1,4-dehydropyridine form) was oxidized by CYP2C76 (Fig. 3A). An $[M+H]^+$ ion of another nifedipine metabolite at *m/z* 345 was also subjected to product ion scan. The spectral pattern of fragment ions was identical to that of the dehydronifedipine standard, indicating that this metabolite was the known dehydronifedipine (Fig. 3B). Bupropion and its metabolite showed different spectral patterns of product ions. The product ion of *m/z* 184 in Figure 3C (a) was considered to be generated by elimination of a *t*-butyl group of bupropion. The fragment ion of *m/z* 238 in Figure 3C (b) was considered to be generated by neutral loss of water from the bupropion metabolite. The latter metabolite has been known to be generated in other species such as humans and rats, and the obtained spectral data were consistent with the previous report (Yeniceli et al., 2011), suggesting that the same metabolite is also generated in monkeys.

Docking simulation

Molecular dockings of nifedipine and bupropion to the modeled CYP2C76 enzyme were investigated (Fig. 4). The methyl carbon that branched from 1,4-dihydropyridine ring of nifedipine was positioned toward the active site of CYP2C76; the ligand-P450 interaction energies (*U* values) and distance from center of the heme were found to be -36.1 kcal/mol and 4.6 Å, respectively (Fig. 4A). This finding suggested that this position of methyl carbon that branched from the 1,4-dihydropyridine ring of nifedipine could become a metabolic site of CYP2C76, consistent with LC-MS/MS analysis shown in Fig. 3A. In bupropion, the methyl carbon of the *t*-butyl group was positioned toward the active site of CYP2C76; *U* values and distance from the center of the heme were found to be -29.2 kcal/mol and 6.5 Å, respectively (Fig. 4B), indicating this position to be a better metabolic site of CYP2C76. These simulation results were consistent with the known structure of bupropion metabolites reported previously (Yeniceli et al., 2011). The estimated structures of nifedipine and bupropion metabolites are shown in Figure 5.

Discussion

Several compounds, including pitavastatin lactone, testosterone, and 7-ethoxyresorufin, have been identified as substrates for CYP2C76; however, a broad evaluation of potential substrates for CYP2C76 has not yet been conducted to understand the substrate specificity of this isoform. In this study, 89 marketed compounds, including human CYP2C and non-CYP2C substrates or inhibitors, were screened as potential substrates for CYP2C76. From the compounds screened, 19 chemicals were identified as substrates for CYP2C76 (Fig. 1A). These structures consisted of the substrates or inhibitors for human P450s listed in Food and Drug Administration Drug-Drug Interaction Draft Guidance, 2006; substrates for CYP1A2 (7-ethoxyresorufin), CYP2B6 (bupropion), CYP2D6 (dextromethorphan), and CYP3A4/5 (dextromethorphan and nifedipine), and inhibitors for CYP2B6 (sertraline, clopidogrel, and ticlopidine), CYP2C8 (quercetin), CYP2C19 (ticlopidine and nootkatone), and CYP3A4/5 (troleandomycin). Of course, limitations exist in the use of the substrate depletion method to identify potential CYP2C76 substrates, i.e. the inability to detect slowly metabolized compounds because of loss of protein activity and difficulty to ascertain true metabolism from experimental noise. As an example, bufuralol was not detected as a substrate for CYP2C76 in this study, but has been identified to be a substrate by examining metabolite formation (Uno et al., 2011b). The intent of this work was not to identify all of the potential CYP2C76 substrates from our list of 89 compounds and some low clearance compounds may have been overlooked; however, several structurally diverse compounds were newly identified in this study.

In this study, monkey CYP2C76 was shown to participate in bupropion hydroxylation and different drug oxidative metabolite(s) formation of nifedipine presumably due to its relatively large substrate cavity with suable substrate orientations. To compare the active

site cavities for CYP2C76 and human CYP3A4, the CYP2C76 homology model was built from human CYP2C9 crystal structure. With binding of nifedipine as a ligand, the cavity size of CYP2C76 was comparable to human CYP3A4 (data not shown). The ability of CYP2C76 to metabolize a wide variety of diverse structures from relatively small molecules (e.g. nootkatone) to larger molecules (e.g. troleandomycin), may be attributed to its wide binding pocket of the active site with suable substrate orientations. Human CYP3A4 has been reported to have a hydrophobic cluster consisting of Phe-213, Phe-214, Phe-219, and Phe-220 in terms of ligand-binding for catalytic function (Ekroos and Sjögren, 2006). Monkey CYP2C76 also had Phe-201, Phe-205, and Phe-294 over the heme around the active site area, similar to human CYP3A4 (data not shown). The total interaction energies (*U* value) of nifedipine with modeled CYP2C76 enzyme were -36.1 kcal/mol (Fig. 4), which was comparable to those with modeled monkey CYP3A4 (-42.9 kcal/mol) and human CYP3A4 (-59.9 kcal/mol). These structural similarities between human CYP3A4 and monkey CYP2C76 might be one of the determinant factors for CYP2C76 to be involved in nifedipine oxidation flexible to accommodate different substrate orientations.

Although most of the CYP2C76 substrates also disappeared in the presence of other monkey CYP2Cs (CYP2C9, and CYP2C19), bupropion and nifedipine showed high selectivity to CYP2C76 (Fig. 1B). Bupropion is oxidized by CYP2B6 in humans and by CYP2B1 in rats, and converted to hydroxybupropion whose molecular weight is 16 Da larger than the parent compound (Coles and Kharasch, 2007; Yeniceli et al., 2011; Pekthong et al., 2012). In monkeys, bupropion was predominantly metabolized by CYP2C76 and CYP2B6 (Table. 2). Whereas, nifedipine is dehydrogenated by CYP3A4 in humans and by CYP2C11 in rats, and converted to a pyridine form whose molecular weight is 2 Da smaller than nifedipine (Guengerich et al., 1986; Shimada et al., 1997; Chovan et al., 2007). In monkeys, nifedipine was predominantly metabolized by CYP3A4 and CYP2C76 (Table. 2). These

differences of P450 isoforms may result in species differences in pharmacokinetic profiles of some drugs.

Importantly, CYP2C76 formed a unique nifedipine metabolite whose molecular weight was 16 Da larger than the parent compound, suggesting addition of a single oxygen atom. This metabolite was not produced by monkey CYP2C9, CYP2C19, or CYP3A4 (Fig. 2A) tested in the present study. Therefore, formation of this unique nifedipine metabolite could be a selective marker reaction of CYP2C76, although more detailed experiments with other monkey P450 isoforms or liver microsomal fractions are needed in the future. To our knowledge, this unique nifedipine metabolite has not been reported in humans. The lines of results indicated that CYP2C76 was able to produce some monkey-specific metabolite(s) of new chemical entities in cynomolgus monkeys. It would be beneficial to consider the presence of CYP2C76 when cynomolgus monkeys are used in preclinical studies. For example, in case some lethal toxicity or other severe problems were observed in monkeys, which were accounted for by CYP2C76 function, one would not have to withdraw the compound from development.

Monkeys are widely used in the preclinical studies to predict bioavailability (BA) in humans; however, they occasionally show a poorer BA than humans (Chiou and Buehler, 2002; Takahashi et al., 2009; Akabane et al., 2010). Among the reported poor-BA compounds were amitriptyline, nifedipine, and imipramine, which were found as CYP2C76 substrates in this study (Fig. 1). Bioavailability is a product of fraction absorbed from gastro-intestinal tract (Fa*Fg) and fraction escaping hepatic elimination (Fh). In the case of amitriptyline, low BA can be attributed to first-pass intestinal metabolism, resulting in low Fa*Fg (Akabane et al., 2010). Lately, P450 of small intestine was quantified in cynomolgus monkeys by immunoblotting; the content of CYP3A was most abundant (about 80% of

immunoquantified total P450 content) similar to humans (Paine et al., 2006; Uehara et al., 2014). Because CYP2C76 was not detected in monkey small intestine (Uehara et al., 2014), CYP2C76 seems to be irrelevant to low Fa*Fg of amitriptyline. On the other hand, nifedipine and imipramine were reported to show low Fh (Takahashi et al., 2009). Considering that CYP2C76 is expressed in the liver of cynomolgus monkeys (4%), albeit to the lower content than CYP3A4 (26%) (Uehara et al., 2011), CYP2C76 might contribute to high hepatic extraction of nifedipine and imipramine.

In conclusion, 19 structurally diverse substrates for CYP2C76 were identified among the 89 substrates evaluated. CYP2C76 has the ability to metabolize a wide variety of diverse structures. Among the newly identified substrates, CYP2C76 formed the unique metabolite of nifedipine, which is possibly a selective marker reaction of CYP2C76. The results revealed that CYP2C76 had relatively wide substrate specificity and the ability to form unique metabolite, which might result in species differences in drug metabolism between monkey and humans. Our findings about substrate specificity of CYP2C76 should help to gain a better understanding of drug metabolism in monkeys and a better interpretation of preclinical data.

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Authorship contribution

Participated in research design: Uno, and Yamazaki.

Conducted experiments: Hosaka, Murayama. Uehara, and Fujino.

Contributed new reagents or analytic tools: Satsukawa,

Performed data analysis: Hosaka, Shimizu, Iwasaki, Iwano, and Uno.

Wrote or contributed to the writing of the manuscript: Hosaka, Iwano, Uno, and Yamazaki.

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Legends for figures

Fig. 1. Substrate disappearance ratio of compounds metabolized by recombinant monkey P450s.

Substrates (1 μM) were incubated with each recombinant P450 for 30 min. The substrates whose disappearance ratio exceeded 20% are shown. Substrate disappearance ratio (%, A) and relative ratio normalized by the results with CYP2C76 in every reactions (B) are shown.

Fig. 2. Chromatographic profile of nifedipine and bupropion after incubation with recombinant monkey P450s.

Substrates (1 μM) were incubated with each recombinant monkey P450 for 0 (control) or 30 min, and the samples were analyzed by positive or negative single MS scan mode. (A) Nifedipine metabolites were analyzed by negative mode at *m/z* 361 for (a) control, (b) CYP2C76, (c) CYP2C9, (d) CYP2C19, and (e) CYP3A4; and positive mode at *m/z* 345 for (a') control, (b') CYP2C76, and (e') CYP3A4. (B) Bupropion metabolites were analyzed in positive mode at *m/z* 256 for (a) control, (b) CYP2C76, (c) CYP2C9, (d) CYP2C19, and (e) CYP2B6

Fig. 3. Comparison of the MS/MS spectra of nifedipine or bupropion to those of their metabolites formed by CYP2C76 or CYP3A4.

Substrates (20 μM) were incubated with CYP2C76 for 30 min, and the samples were analyzed in positive or negative product ion scan mode. (A) Nifedipine and its oxdative metabolite were analyzed (scan mode: negative, scan range: *m/z* 50-365, and collision energy: 15 V). (B) Dehydronifedipine and nifedipine metabolites were analyzed (scan mode:

positive, scan range: *m/z* 50-365, and collision energy: 45 V). (C) Bupropion and its oxidative metabolite were analyzed (scan mode: positive, scan range: *m/z* 50-260, and collision energy: 15 V).

Fig. 4. Docking simulation of the interaction of nifedipine and bupropion with modeled CYP2C76 enzyme.

Oxygen, nitrogen, and iron atoms are colored with red, blue, and light blue, respectively. The total interaction energies (*U* value) of nifedipine (A) and bupropion (B) with modeled CYP2C76 enzyme were -36.1 kcal/mol and -29.2 kcal/mol, respectively. The distances between metabolism sites of nifedipine (A) and bupropion (B) to heme iron were 4.6 Å and 6.5 Å, respectively.

Fig. 5. Putative structure of nifedipine and bupropion metabolite.

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Table 1. Eighty-nine compounds evaluated in this study

Amiodarone	Erythromycin	Nifedipine	Sulfadiazine
Amitriptyline	7-Ethoxyresorufin	p-Nitrophenol	Sulfamethoxazole
Amlodipine	Fluconazole	Nootkatone	Sulfaphenazole
Amodiaquin	Fluoxetine	Olanzapine	Tacrine
Amoxapine	Flurbiprofen	Omeprazole	Taxol
Atorvastatin	Fluvastatin	Orphenadrine	Terfenadine
Azamulin	Fluvoxamine	Phenacetin	Testosterone
Bufuralol	Furafylline	Phenylbutazone	Theophylline
Bupropion	Gemfibrozil	Pioglitazone	Thiotepa
Caffeine	Ifosfamide	Piroxicam	Ticlopidine
Chlormethiazole	Imipramine	Pitavastatin	Tolbutamide
Chlorzoxazone	Irsogladine	Pitavastatin lactone	Torsemide
Citalopram	Itraconazole	Pravastatin	Tranylcypromine
Clomipramine	Ketokonazole	Proguanil	Triazolam
Clopidogrel	Lansoprazole	Propranolol	Trimethoprim
Clozapine	Loratadine	Quercetin	Troglitazone
Coumarin	Maprotiline	Quinidine	Troleandomycin
Cyclophosphamide	Methoxsalen	Ritonavir	Tryptamine
Dapson	Miconazole	Rosiglitazone	Verapamil
Dextromethorphan	Midazolam	Rosuvastatin	Warfarin
Diazepam	Montelukast	Salbutamol	
Diclofenac	α-Naphthoflavone	Sertraline	
Efavirenz	Nicardipine	Simvastatin	

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Table 2. CL_{int} values for CYP2C76 substrates

Compound	CL_{int} (mL/min/nmol P450)				
	CYP2C9	CYP2C19	CYP2C76	CYP3A4	CYP2B6
Pitavastatin lactone[a]	3.36	0.85	0.43	N.D.	N.D.
Nifedipine	0.67	0.23	4.23	6.57	N.D.
Bupropion	<0.10	<0.10	0.47	N.D.	0.19

N.D., not determined

[a] Pitavastatin lactone was used as positive control because CYP2C76 is important for monkey-specific metabolism of pitavastatin lactone (Uno et al., 2007).

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Fig. 1

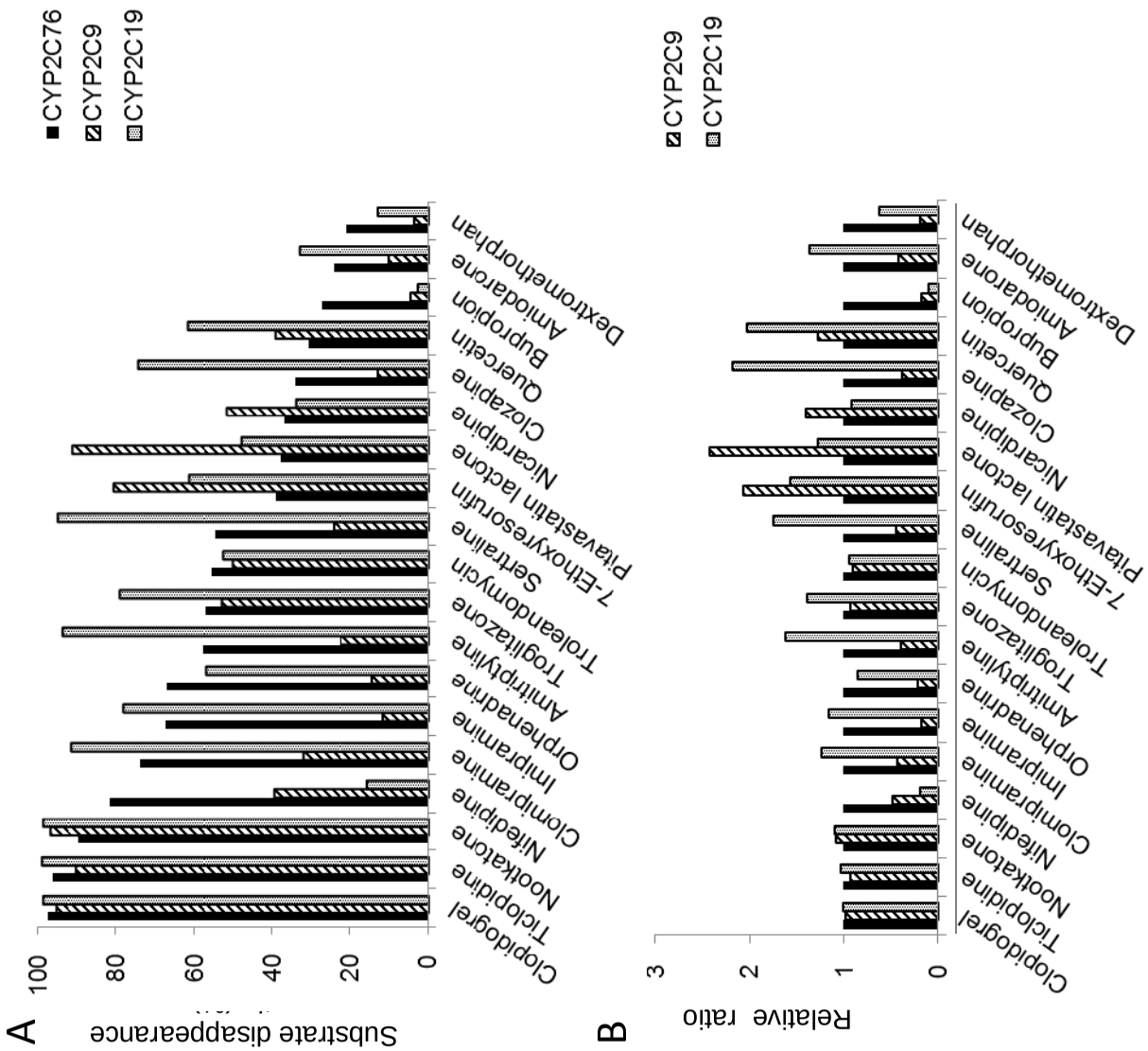

Fig. 2

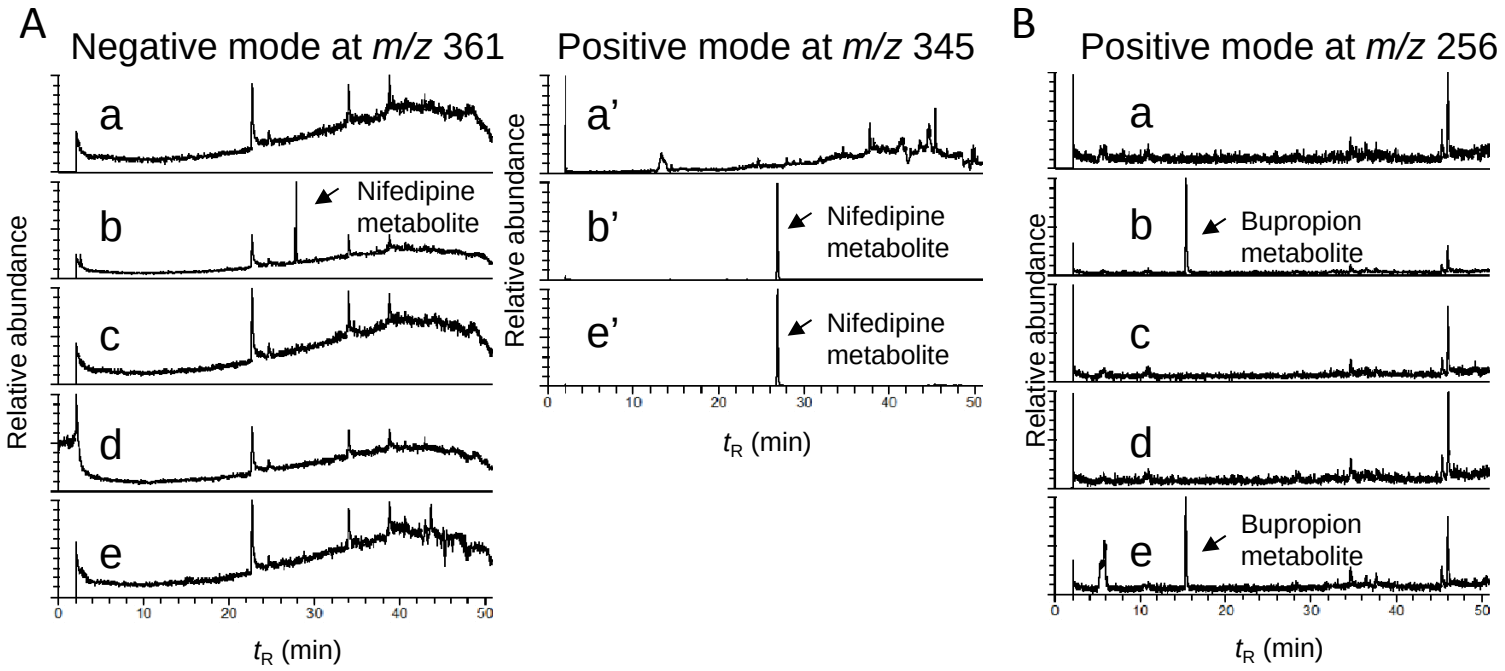

Fig. 3

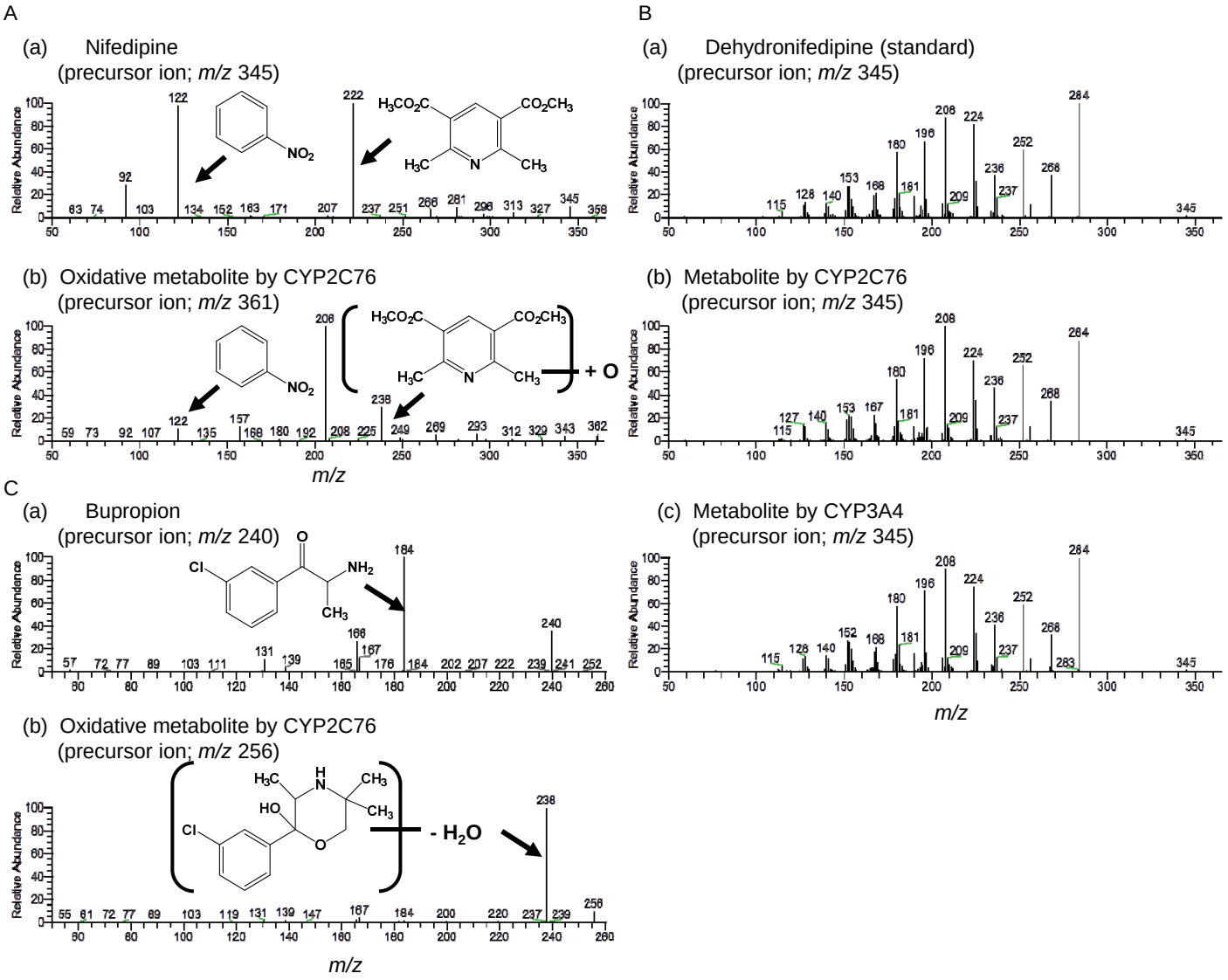

Fig. 4

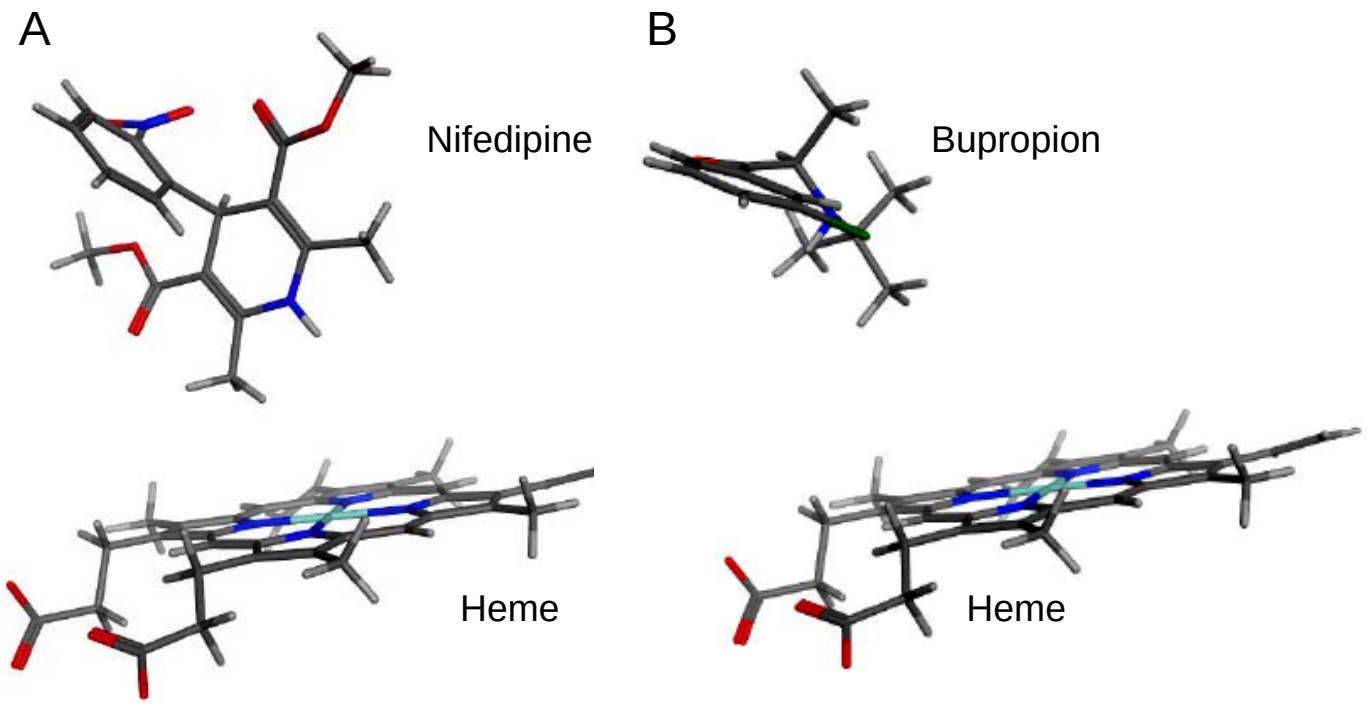

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Fig. 5